# Seventeen Complementation Groups of Mutations Decreasing Meiotic Recombination in *Schizosaccharomyces pombe*

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Manuscript received August 5, 1991
Accepted for publication September 30, 1991

ABSTRACT

We have analyzed 43 recessive mutations reducing meiotic intragenic recombination in *Schizosaccharomyces pombe*. These mutations were isolated by a screen for reduced plasmid-by-chromosome recombination at the *ade6* locus. Sixteen of the mutations define 10 new complementation groups, bringing to 17 the number of genes identified to be involved in meiotic recombination. The mutations were grouped into three discrete classes depending on the severity of the recombination deficiency in crosses involving the *ade6-M26* recombination hotspot. Class I mutations caused at least a 1000-fold reduction in *M26*-stimulated intragenic recombination at the *ade6* locus. Class II mutations reduced *M26*-stimulated recombination approximately 100-fold. Class III mutations caused a 3–10-fold reduction in either *M26*-stimulated or non-hotspot recombination. We obtained multiple alleles of class I and class II mutations, suggesting that we may be nearing saturation for mutations of this type. As a first step toward mapping, we used mitotic segregation to assign fourteen of the *rec* genes to chromosomes. Mutations in the six *rec* genes tested also caused a decrease in intragenic recombination at the *ura4* locus; five of these mutations also reduced intergenic recombination between the *pro2* and *arg3* genes. These results indicate that these multiple *rec* gene products are required for high level meiotic recombination throughout the *S. pombe* genome.

BOTH biochemical and genetic approaches have been used to investigate the mechanism of genetic recombination in eukaryotes. Biochemical analyses involving the purification of activities predicted to be necessary for recombination are limited in that usually only those activities that are assayed will be found. Eventually, a mutant lacking this activity must be obtained to determine whether or not the activity is indeed involved in recombination in the cell. A genetic approach has the advantage of producing the mutants with fewer constraints imposed by the imagination of the investigator. An experimental design that combines the two approaches is the ultimate goal. With this end in mind, we have undertaken a continuation of the work initiated by PONTICELLI and SMITH (1989) to identify genes involved in meiotic recombination in *Schizosaccharomyces pombe* through the isolation and analysis of recombination-deficient mutants.

Prior to the work of PONTICELLI and SMITH (1989) only a few *S. pombe* mutations affecting meiotic recombination had been isolated. A dominant mutation in a gene, *rec1*, reduces mitotic recombination at the *ade6* locus but does not alter the meiotic recombination rate (GOLDMAN and GUTZ 1974). Three recessive mutations (*rec2*, *rec3* and *rec5*) isolated on the basis of reduced ectopic recombination between the unlinked tRNA suppressor genes, *sup3*, *sup9* and *sup12*, have no effect on general meiotic recombination, suggesting their involvement in illegitimate pairing (THURIAUX 1985). Mutations in 10 *swi* genes involved in mating-type switching were analyzed, but mutations in only one, *swi5*, show a deficiency in meiotic recombination (GUTZ and SCHMIDT 1985; SCHMIDT, KAPITZA and GUTZ 1987).

PONTICELLI and SMITH (1989) developed a screen that identified multiple mutants deficient in meiotic recombination. They isolated mutants with a decreased frequency of recombination between two alleles of the *ade6* gene, one carried on a multicopy plasmid and the other in the normal chromosomal location. Since recombination is necessary for the proper segregation of chromosomes at meiosis I, mutants deficient in recombination have reduced spore viability due to frequent aneuploidy; however, the three chromosomes of *S. pombe* assorting randomly will produce a true haploid approximately 10% of the time, allowing sufficient viability for measurement of recombinant frequencies. Colonies of mutagenized cells were therefore screened for a decrease in the number of $Ade^+$ prototrophs produced in a meiotic homothallic self-cross, which allowed the isolation of recessive mutations. They isolated thirteen mutations, one of which was dominant. Of the recessive mutations, nine defined six complementation groups (*rec6–rec11*), and three were not analyzed further. Mutations in five of the six *rec* complementation groups decrease recombination at least 100-fold, whereas the remaining mutation decreases recombination only

TABLE 1

*S. pombe* strains

| Strain | Genotype | Source or reference[a] |
|---|---|---|
| GP6 | *h⁺ ade6-M375* | (1) |
| GP13 | *h⁻ ade6-52* | (2) |
| GP18 | *h⁻leu1-32* | V. ZAKIAN |
| GP24 | *h⁺ ade6-M26* | (1) |
| GP27 | *h⁺ ade6-M210 sup9* | (1) |
| GP43 | *h⁻ ade6-M375 leu1-32* | GP6 × GP18 |
| GP66 | *h⁹⁰ ade6-M26 ura4-294* (pade6-469) | (2) |
| GP213 | *h⁹⁰ swi5-134 lys2 ade6-M210* | J. KOHLI |
| GP349 | *h⁻ arg3-124 ura4-294* | (2) |
| GP350 | *h⁺ pro2-1 ura4-595* | (2) |
| GP351 | *h⁻ pro2-1 ura4-595* | This study[b] |
| GP363 | *h⁺ ade6-M26 ura4-294 arg3-124* | (2) |
| GP364 | *h⁺ ade6-M26 ura4-294 arg3-124 rec6-103* | GP349 × GP275[c] |
| GP366 | *h⁺ ade6-M26 ura4-294 arg3-124 rec9-104* | GP349 × GP312[c] |
| GP369 | *h⁻ ade6-52 ura4-595 pro2-1* | (2) |
| GP370 | *h⁻ ade6-52 ura4-595 pro2-1 rec6-103* | GP350 × GP273[d] |
| GP372 | *h⁻ ade6-52 ura4-595 pro2-1 rec9-104* | GP350 × GP288[d] |
| GP427 | *h⁺ ade6-52 ura4-595 pro2-1 rec8-110* | GP350 × GP290[d] |
| GP436 | *h⁻ ade6-M26 ura4-294 arg3-124 rec8-110* | GP349 × GP316[c] |
| GP500 | *h⁺ ade6-M26 ura4-294 arg3-124 rec12-117* | GP363 × GP497[d] |
| GP501 | *h⁺ ade6-M26 ura4-294 arg3-124 rec13-119* | GP363 × GP498[d] |
| GP502 | *h⁺ ade6-M26 ura4-294 arg3-124 rec14-120* | GP363 × GP499[d] |
| GP503 | *h⁻ ade6-52 ura4-595 pro2-1 rec12-117* | GP350 × GP497 |
| GP504 | *h⁻ ade6-52 ura4-595 pro2-1 rec13-119* | GP350 × GP498 |
| GP505 | *h⁻ ade6-52 ura4-595 pro2-1 rec14-120* | GP350 × GP499 |
| GP605 | *h⁹⁰ mat2-B102 ade6-52 his5-303 leu2-120* | This study[b] |
| GP699 | *h⁻ ade6-52 pro2-1 ura4-595 rec8-110* | GP351 × GP426[e] |

[a] (a) PONTICELLI, SENA and SMITH (1988); (2) PONTICELLI and SMITH (1989).
[b] Genealogy is available upon request.
[c] This strain is described in reference (2).
[d] See Table 2 for genotype. The derivation is described in the text.
[e] GP426, isolated from the same cross as GP427, has the same genotype.

threefold. Since only one or two alleles of each gene was isolated, the pool of *rec* genes in *S. pombe* was far from saturated by this mutagenesis. We have continued this work by isolating and analyzing more mutants using the same screen. Twenty-seven new mutants, in addition to the three mutants not further analyzed by PONTICELLI and SMITH (1989), fall into 16 complementation groups, 10 of which have not been described previously. Several alleles of the strongest *rec* mutations were isolated, which suggests that we are now approaching saturation for these genes.

## MATERIALS AND METHODS

**Strains:** *S. pombe* strains are described in Table 1 and other tables where appropriate, following the genetic nomenclature of KOHLI (1987).

**Plasmids and transformation:** Plasmid DNA was introduced into *S. pombe* strains using the LiOAc method of ITO *et al.* (1983). Plasmid pade6-469 contains the *ade6-469* allele and the *Saccharomyces cerevisiae URA3* gene (SZANKASI *et al.* 1988). The *URA3* gene, when present in high copy, complements the uracil requirement of an *S. pombe ura4* mutation.

***S. pombe* culture media:** Rich medium was yeast extract agar (YEA) or yeast extract liquid (YEL) (GUTZ *et al.* 1974). Synthetic sporulation agar (SPA) and malt extract agar (MEA) were prepared according to GUTZ *et al.* (1974). Minimal media used were MMA (GUTZ *et al.* 1974) and NBA (PONTICELLI and SMITH 1989). Liquid minimal medium (NBL) was NBA without agar. SPA, MEA, MMA and NBA were supplemented with required amino acids, purines and pyrimidines at 100 μg/ml.

**Isolation of *rec* mutants:** We used the screen of PONTICELLI and SMITH (1989) to isolate additional mutants deficient in meiotic plasmid-by-chromosome recombination. Briefly, homothallic strain GP66, which carries the chromosomal *ade6-M26* and *ura4-294* mutations and the plasmid pade6-469, was mutagenized with nitrosoguanidine and plated on minimal MMA medium with adenine but without uracil, to maintain selection for the plasmid. After 5 days of incubation, many cells had mated and sporulated. Individual colonies were transferred to microtiter wells, treated with glusulase and ethanol, and spotted onto MMA + uracil or onto YEA. Typically, unmutagenized GP66 gave approximately 50 white ($Ade^+$) papillae on YEA, whereas *rec* candidates gave few or no papillae on YEA, and few or no $Ade^+$ colonies on MMA + uracil.

Each candidate was grown nonselectively in rich medium to obtain $Ura^-$ segregants which had lost pade6-469. To eliminate the possibility that the recombination deficiency was due to a plasmid mutation, pade6-469 was reintroduced into several candidates and the plasmid-by-chromosome recombinant frequency redetermined. Since this test did not eliminate any candidates in the first experiment, it was not used in later experiments unless *rec* derivatives were difficult to isolate after meiotic crosses. To test for additional mutations in the *ade6* gene, some of the $Ura^-$ segregants were crossed with a strain (GP27) carrying the *sup9* mutation, which suppresses the adenine requirement of the *ade6-M26* mutation (GUTZ 1971). To test for dominance or recessiveness of the *rec* mutations in chromosome-by-chromosome recombination, each candidate was also crossed with a $rec^+$ strain (GP13).

**Meiotic crosses:** For crosses of two heterothallic strains, equal volumes of saturated cultures in YEL were mixed in a 1.5-ml microfuge tube, the cells were washed with saline, and the entire sample, in approximately 10 μl of saline, was spotted on supplemented SPA medium. When one strain was homothallic, that strain was used at one-fifth the volume of the heterothallic strain to increase the frequency of interstrain matings. After incubation for 3–5 days at room temperature, the matings were harvested into 0.5 ml of 0.5% glusulase in water and incubated overnight at room temperature. The spore suspensions were then treated with 30% ethanol for 30 min and washed once with water prior to plating.

When testing a large number of isolates in test crosses, matings were done on MEA as "cross stamps." Candidates to be tested were patched on YEA plates, 32 per plate, and allowed to grow overnight. The strains to be mated with the candidates were spread from a saturated culture onto YEA plates and also grown overnight. The YEA plate with the patched candidates was replicated onto supplemented MEA, and the second parent applied to the MEA plate with a sterile wooden applicator (a tongue depressor cut in half crosswise) in a line perpendicular to and intersecting the

patched strains. The mating plate was incubated at room temperature for 3–5 days. When the test crosses were performed to determine mating type, the mating plate was exposed to iodine vapors, which stain sporulating colonies (GUTZ *et al.* 1974). For determination of the *rec* phenotype, the mating plate was replicated onto an NBA plate without adenine. The NBA plate was incubated for two days and replicated onto an identical NBA plate, which was incubated for 2 days more and scored for growth of Ade$^+$ recombinants. We found this sequential replication to be critical for accurate scoring of the *rec* phenotype.

**Determination of meiotic recombinant frequencies:** On media, such as YEA, that contain limiting amounts of adenine, *S. pombe* strains carrying the *ade6-M26* or *ade6-M375* allele are dark red, whereas *ade6-52*-carrying strains are pink (GUTZ *et al.* 1974). Ade$^+$ colonies are white under these conditions. To measure plasmid-by-chromosome recombination, the homothallic *ade6-M26 rec* strains carrying pade6-469 were allowed to self-mate. The spore suspension was diluted and plated on YEA to determine total viable spores and, in some cases, Ade$^+$ (white) recombinants. In some experiments, Ade$^+$ recombinants were also selected on NBA plates.

For quantitation of chromosome-by-chromosome recombination, two strains were grown in YEL and crossed as described above. The spore suspension was diluted and plated on YEA to determine total viable spores and on NBA to select for prototrophic recombinants.

In order to determine whether the Ade$^+$ colonies in *rec* mutant crosses were true recombinants or simply revertants of the *ade6* mutations, we measured the meiotic revertant frequencies of the three chromosomal *ade6* alleles used. For each allele, crosses were performed between an $h^+$ *ade6* and an $h^-$ *ade6* strain, and the spore suspensions plated as described above for determination of recombinant frequencies. Each allele was tested eight times, and the strains for each trial were grown in separate cultures from individual single colonies. The revertant frequencies were as follows (in Ade$^+$/$10^6$ viable spores): *ade6-52*, 0.11, <0.76, <0.3, <0.23, <0.19, <0.25, <0.13, <0.2; *ade6-M26*, 2.1, 0.18, <0.47, <3.5, <0.22, <0.45, <0.37, <0.27; and *ade6-M375*, <0.14, 0.67, <0.22, <0.48, <0.29, <0.27, <0.33, <0.29. In those cases where no Ade$^+$ revertants were seen, the upper limits of the revertant frequencies were calculated based on an assumption of three Ade$^+$ revertants in the sample plated.

**Complementation of *rec* mutations:** Complementation of *rec* mutations was determined first qualitatively, in a "spot" test, and then quantitatively as described above for measurement of *ade6* chromosome-by-chromosome recombinant frequency. In the "spot" test, strains to be tested were grown in YEL, mixed in a microfuge tube and spotted on SPA sporulation medium. Instead of harvesting the mating, the SPA plate was replicated onto an NBA + uracil plate which was incubated for two days, and then replicated onto an identical NBA + uracil plate, which was incubated for 2 days more. Comparison of the Ade$^+$ recombinants present in each experimental mating to control matings allowed qualitative assignment of wild-type or mutant levels of recombination. This qualitative test decreased significantly the number of quantitative crosses necessary to accurately assign complementation groups.

**Mitotic mapping by formation of stable diploids and induction of haploidization:** Strains with the genotype $h^{90}$ *mat2-B102* mate with either heterothallic type ($h^+$ or $h^-$) but fail to sporulate when crossed with an $h^-$ strain (EGEL 1984). We employed this phenotype to create stable diploids, modifying the procedure of KOHLI *et al.* (1977). An auxotrophic $h^{90}$ *mat2-B102* strain was crossed on SPA with an $h^-$ strain carrying different auxotrophies. After 20 hr at room temperature, the mating mixture was scraped with a toothpick and streaked onto NBA to select for protrophic diploids. After three days at 32°, large colonies were individually inoculated into 1 ml of supplemented NBL containing 0.4–0.8% *m*-fluorophenylalanine to induce haploidization (KOHLI *et al.* 1977). After 2 days at 32°, the cultures were diluted and plated on YEA. The YEA plates were incubated for 2 days and replicated onto NBA + adenine. Each parent contained, in addition to other markers, an auxotrophy on chromosome *I* (either *leu2* or *pro2*). Thus, haploids were identified as auxotrophs. By this criterion, 50–95% of the colonies were haploids. Haploids were picked from the YEA plate onto another YEA plate in a grid, which in turn was used for replication onto minimal plates to score the auxotrophies or onto MEA plates to score for *rec*, as described above.

**Sensitivity to DNA damaging agents:** To test for sensitivity to MMS (methyl methane sulfonate), strains were streaked on YEA medium containing MMS (50–100 μl/liter) and 0.1% (wt/vol) caffeine. Sensitivity to UV (ultraviolet light) was determined qualitatively by spotting 10 μl of a saturated culture in YEL onto a YEA plate, exposing it to up to 3200 ergs/mm$^2$ UV, and incubating the plate at 32° for 3 days. Sensitivity was scored as either reduced colony size or failure to grow.

## RESULTS

**Isolation of additional *rec* mutants:** We used the screen described above to isolate mutants defective in meiotic *ade6* plasmid-by-chromosome recombination, which were then examined for chromosome-by-chromosome recombination deficiencies. This screen takes advantage of the recombination "hotspot" activity of the *ade6-M26* allele (GUTZ 1971), which stimulates recombination at the *ade6* locus 10–15-fold, thus providing a higher initial recombinant frequency from which to screen for recombination-deficient mutants. Because the starting strain was homothallic and was allowed to self-mate, recessive mutations were recovered.

Five independent cultures of strain GP66, grown from single colonies, were mutagenized. Approximately 18,000 colonies were screened, and eighty-two candidates were chosen that appeared in the initial screen to have a recombination deficiency. For six of these, Ura$^-$ segregants could not be isolated, and they were discarded. Of the remaining 76, 36 strains carried dominant mutations; 8 of these were tested for additional *ade6* mutations by crossing with a *sup9* strain, which suppresses the adenine requirement of the *ade6-M26* mutation present in the parental strain. Five of the eight were no longer suppressible, suggesting that the majority of the dominant mutations were additional mutations in the *ade6* gene. Strains carrying dominant mutations were not characterized further in this study. The remaining forty strains carried recessive mutations, and their Ura$^-$ segregants were used for further analysis. In addition, three mutants (*rec-106*, *rec-112* and *rec-113*) isolated by

PONTICELLI and SMITH (1989) were further characterized in this study, for a total of forty-three recessive mutations.

**Identification of 10 new complementation groups and additional alleles of defined groups:** Seven recessive mutants from the first two mutageneses were tested for complementation with *rec6–rec11* by crossing each $h^{90}$ *ade6-M26 ura4-294 rec* candidate with $h^-$ *ade6-52* strains carrying *rec6–rec11* and quantitating the recombinant frequency (data not shown). Two mutants carried new alleles of *rec8* and *rec10* and are discussed below. Five mutants defined new complementation groups: *rec12*, *rec13*, *rec14*, *rec15* and *rec16*. The $h^-$ *ade6-52 rec* derivatives of the *rec12–rec16* strains were obtained for further analysis.

Ura$^-$ segregants of the remaining thirty-six new mutants (including *rec-106*, *rec-112* and *rec-113*) were crossed with the $h^-$ *ade6-52* derivatives of the *rec6–rec16* strains and analyzed by the spot complementation test. Eighteen mutants appeared to complement *rec6–rec16* in these homothallic-by-heterothallic spot tests and were mated with GP13 to isolate $h^-$ *ade6-52 rec* derivatives. For only 5 of the 18 mutants were $h^-$ *ade6-52 rec* derivatives obtained; the remaining 13 mutants were not analyzed further. The appropriate derivatives of these five mutants were crossed against each other: each represented a different complementation group, thus defining *rec17*, *rec18*, *rec19*, *rec20* and *rec21*. For *rec12–rec21*, the $h^-$ *ade6-52 rec* strains were crossed with an $h^+$ *ade6-M26* strain (GP24), and the $h^+$ *ade6-M26 rec* derivatives were isolated. These $h^-$ *ade6-52 rec* and $h^+$ *ade6-M26 rec* derivatives of each new mutant (*rec12–rec21*) were used to verify complementation with the existing *rec* mutants (*rec6–rec11*) and with each other in heterothallic-by-heterothallic crosses (Table 2). All of the new mutations complemented the recombination deficiency of the *swi5–134* strain, which reduces *ade6* chromosome-by-chromosome recombination approximately 10-fold (Table 2; SCHMIDT, KAPITZA and GUTZ 1987). These results brought to 17 (including *swi5*) the number of complementation groups affecting meiotic *ade6* interchromosomal recombination.

We isolated additional alleles of several of the known complementation groups. Twenty of the original 43 recessive mutants failed to complement one of the *rec6–rec21* mutations. Table 3 shows the results of crosses in which the homothallic Ura$^-$ segregants containing the new *rec* alleles were mated with the heterothallic derivatives of two known *rec* mutants: one which failed to complement, to verify the group assignment, and one which complemented, to verify recessiveness. In all but one case, the new alleles showed a recombinant frequency comparable to the original allele. *rec6-130* showed a repeatedly higher recombinant frequency than the original allele (*rec6-103*) and the other new *rec6* alleles (*rec6-126* and *rec6-137*). *rec6-130* apparently has a "leaky" phenotype. It should be noted that *rec6-126* and *rec6-130* came from the same mutagenized culture. However, we believe that these alleles are not clonally related since they show distinctly different recombination phenotypes. *rec15-124* and *rec15-127* were also isolated from the same mutagenized culture, but we believe that these two alleles are independent, since the original *rec15-124* isolate also carried a mutation which caused low spore yields. Two of the mutations (*rec-106* and *rec-113*) described by PONTICELLI and SMITH (1989) are alleles of *rec11*; their third unassigned mutation (*rec-112*) defined the *rec17* complementation group. One allele from each new complementation group was chosen for further characterization in this study. The number of alleles for each of the complementation groups is discussed below.

**Characterization of *rec* deficiencies:** In crosses homozygous for a given *rec* mutation (Table 2) the reduction in chromosome-by-chromosome recombination varied from approximately threefold (*rec17* and *rec18*) to approximately 1000-fold (*rec12*, *rec14* and *rec15*). Based on our reversion analysis (see MATERIALS AND METHODS) we believe that the few Ade$^+$ colonies seen in the crosses of the most recombination-deficient mutants represent recombinants and not revertants of either *ade6* allele. Recombinant frequencies not significantly different from those of *rec*$^+$ strains were obtained in crosses where complementation occurred, verifying that these mutations were fully recessive.

**Effect of new mutations on *M26* recombination stimulation:** Since the mutant screen took advantage of the hotspot activity of the *ade6-M26* allele, it was important to compare the effect of each *rec* mutation on hotspot and non-hotspot recombination. The *ade6-M375* mutation is located three nucleotides from *ade6-M26* and is also a single base-pair change of G:C → T:A (SZANKASI *et al.* 1988; PONTICELLI, SENA and SMITH 1988). *ade6-M375*, however, does not show the stimulation of recombination of *ade6-M26* (GUTZ 1971), and thus serves as an excellent control for the *M26* hotspot activity. The $h^+$ *ade6-M375 rec* derivative of each new *rec* mutation was isolated by crossing the $h^-$ *ade6-52 rec* strains with GP6 ($h^+$ *ade6-M375*). The effect of each *rec* mutation on *M26* stimulation of meiotic *ade6* interchromosomal recombination was examined by comparing the recombinant frequency in *ade6-M26* × *ade6-52* crosses with those in *ade6-M375* × *ade6-52* crosses. For each *rec* mutation the recombinant frequency was decreased in both the *M26* and the *M375* crosses (Table 4). In cases where there was a detectable but low level of recombination (*rec12*, *rec14* and *rec15*), both *M26* and *M375* recombinant frequencies had the same absolute value.

**TABLE 2**

**Complementation analysis of new recessive *rec* mutations**

| | | Ade$^+$ recombinants/$10^6$ viable spores | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *rec* | GP No. | *12-117* 592 | *13-119* 593 | *14-120* 599 | *15-124* 594 | *16-125* 595 | *17-112* 687 | *18-138* 661 | *19-139* 662 | *20-144* 729 | *21-143* 691 | *swi5-134* 584 |
| + | 13 | 1000 | 1900 | 1400 | 2300 | 1400 | 1300 | 1800 | 2300 | 1100 | 1900 | 1300 |
| *6-103* | 273 | 1300 | 2600 | 1800 | 1600 | 1000 | 1200 | 1900 | 3000 | 2200 | 1200 | 1100 |
| *7-102* | 277 | 1500 | 1800 | 2300 | 2300 | 800 | 1600 | 2000 | 2000 | 3400 | 1000 | 2100 |
| *8-110* | 290 | 1100 | 1800 | 1300 | 1800 | 1300 | 1200 | 900 | 2700 | 1500 | 1100 | 1300 |
| *9-104* | 288 | 1300 | 1800 | 2500 | 1800 | 2000 | 1500 | 3100 | 2100 | 1300 | 1700 | 1600 |
| *10-109* | 289 | 1100 | 1100 | 800 | 1300 | 1000 | 1000 | 1100 | 1800 | 1700 | 1200 | 1600 |
| *11-111* | 291 | 2100 | 1500 | 1000 | 1300 | 900 | 900 | 1400 | 2400 | 1300 | 1600 | 1600 |
| *12-117* | 497 | **4**[a] | 1500 | 1100 | 1300 | 1600 | 1000 | 800 | 2100 | 1700 | 1400 | 1400 |
| *13-119* | 498 | 1200 | **240** | 1300 | 1600 | 1000 | 1100 | 2000 | 1400 | 1100 | 1000 | 1100 |
| *14-120* | 499 | 1500 | 1900 | **3**[b] | 1600 | 1900 | 1400 | 800 | 2100 | 1300 | 1500 | 1100 |
| *15-124* | 571 | 1600 | 1500 | 1700 | **1**[b] | 1200 | 1700 | 1300 | 1400 | 1600 | 1700 | 2500 |
| *16-125* | 572 | 1100 | 1500 | 1900 | 1500 | **30**[c] | 1000 | 1800 | 2700 | 1300 | 1300 | 1000 |
| *17-112* | 606 | 2000 | 2100 | 2000 | 3200 | 2200 | **450** | 1500 | 2000 | 1900 | 1400 | 1500 |
| *18-138* | 659 | 1700 | 1800 | 1400 | 3100 | 900 | 1000 | **630** | 960 | 3000 | 1700 | 2600 |
| *19-139* | 660 | 900 | 2100 | 2500 | 2900 | 1400 | 1500 | 2800 | **260** | 2000 | 1700 | 1600 |
| *20-144* | 728 | 970 | 700 | 1800 | 1300 | 2000 | 2200 | 1900 | 2300 | **280** | 2000 | 1200 |
| *21-143* | 690 | 2300 | 1300 | 1600 | 2000 | 1300 | 2000 | 1700 | 3000 | 1600 | **400** | 1400 |
| *swi5-134* | 583 | 1300 | 1500 | 1200 | 2000 | 1200 | 1300 | 1800 | 2500 | 1600 | 1200 | **250** |

Strains along the top row were of the genotype $h^+$ *ade6-M26 rec*; strains on the left column were of the genotype $h^-$ *ade6-52 rec*. For *rec6–rec11*, these strains are described by PONTICELLI and SMITH (1989). For *rec12–21*, the derivations of the $h^-$ *ade6-52 rec* and $h^+$ *ade6-M26 rec* strains are described in the text. The *swi5* strains were similarly constructed from GP213 (Table 1). >200 Ade$^+$ recombinants were scored for each recombinant frequency determination, except where noted. >150 colonies (and generally >200, but with 5 exceptions of >115 colonies) were counted to determine total viable spores.

[a] Only 2 Ade$^+$ recombinants were obtained.

[b] Only 1 Ade$^+$ recombinant was obtained.

[c] Only 3 Ade$^+$ recombinants were obtained in this experiment (see Table 4).

**Effect of *rec6*, *rec8*, *rec9*, *rec12*, *rec13* and *rec14* on recombination at other intervals:** PONTICELLI and SMITH (1989) reported that mutations in *rec7*, *rec10* and *rec11* reduce intragenic recombination at the *ura4* locus, as well as intergenic recombination between *pro2* and *arg3*. We determined the effect of their *rec6*, *rec8* and *rec9* mutations, as well as the new *rec* mutations, *rec12*, *rec13* and *rec14*, on recombination at loci other than *ade6* by constructing strains that would allow determination of both *ura4* and *ade6* intragenic and *pro2-arg3* intergenic recombination in the same cross. The data presented in Table 5 show that most *rec* mutations tested decreased both Ura$^+$ and Arg$^+$ Pro$^+$ recombinant frequencies, although the relative reductions differed from locus to locus, as was described for *rec7*, *rec10* and *rec11* (PONTICELLI and SMITH 1989) The exception was *rec8*, which showed strongly decreased intragenic recombination at both *ade6* and *ura4*, but nearly wild-type levels of intergenic recombination at *pro2-arg3* (see DISCUSSION).

**Mitotic phenotypes of the *rec* mutants:** Some mutations affecting meiotic recombination in other organisms have been isolated by their effect on mitotic growth, typically sensitivity to DNA-damaging agents (GAME *et al.* 1980). In contrast, only two of the *S. pombe rec* mutants previously reported (*rec9* and *swi5*) demonstrate any mitotic phenotype (PONTICELLI and SMITH 1989; SCHMIDT, KAPITZA and GUTZ 1987; SCHMIDT *et al.* 1989). Several *rad* mutants of *S. pombe* that show a mitotic sensitivity to UV are not deficient in meiotic intragenic recombination (GROSSENBACHER-GRUNDER and THURIAUX 1981). We tested the effect of UV and MMS + caffeine on mitotically growing strains carrying mutations in *rec12–rec22*. Strains carrying either a *rec17* or a *rec19* mutation showed sensitivity to MMS when combined with caffeine, whereas strains carrying the remaining *rec* mutations did not (data not shown). In addition, cells of *rec17* strains grown mitotically tended to clump in liquid medium. Strains carrying either a *rec17* or a *rec18* mutation had greater doubling times than *rec*$^+$ strains, whereas *rec19* strains were not affected. All three mutations, like *rec9* and *swi5*, only moderately reduced the *ade6* recombinant frequency (3–10-fold). Since each of these complementation groups is represented by only one allele (with the exception of *swi5*, no alleles of which were isolated by this screen) and extensive analysis of meiotic segregants for coinheritance of the mitotic and *rec* phenotypes has not been done, the two phenotypes may be due to separate mutations.

**Linkage group assignments:** As a first step in mapping the 16 *rec* genes, we determined the chromosome bearing each *rec* gene. By constructing multiply marked diploids which also carried the *rec* gene in question and analyzing haploids formed after random chromosome loss, we were able to determine with which chromosomal marker the *rec* gene segregated mitotically.

TABLE 3

**Assignment of new *rec* alleles to complementation groups**

| | | Ade$^+$ recombinants/$10^6$ viable spores | |
|---|---|---|---|
| $h^{90}$ *ade6-M26 ura4-294* | | $h^-$ *ade6-52* | |
| | Strain # | *rec6-103* | *rec7-102* |
| *rec6-103*[a] | GP245 | <27 | 2000 |
| *rec6-126*[b] | GP629 | <8 | 1500 |
| *rec6-130*[b] | GP637 | 140[c] | 4500 |
| *rec6-127* | GP651 | <89 | 880 |
| | | *rec7-102* | *rec6-103* |
| *rec7-102*[a] | GP249 | <58 | 3800 |
| *rec7-129*[b] | GP635 | <23 | 2000 |
| *rec7-131*[b] | GP639 | <78 | 1900 |
| | | *rec8-110* | *rec6-103* |
| *rec8-110*[a,b] | GP258 | 35[d] | 1400 |
| *rec8-118*[b] | GP577 | <59 | 1200 |
| *rec8-135* | GP647 | <8 | 1500 |
| | | *rec10-109* | *rec6-103* |
| *rec10-109*[a,b] | GP257 | 20 | 1500 |
| *rec10-116*[b] | GP576 | 26 | 950 |
| *rec10-133* | GP643 | 13 | 1300 |
| *rec10-134* | GP645 | 79 | 1300 |
| *rec10-136* | GP649 | 21 | 1400 |
| | | *rec11-111* | *rec6-103* |
| *rec11-111*[a,b] | GP259 | 1 | 850 |
| *rec11-106*[b] | GP253 | <320[e] | 900 |
| *rec11-113*[b] | GP264 | <22 | 1200 |
| *rec11-128* | GP633 | | 1400 |
| | | *rec12-117* | *rec6-103* |
| *rec12-117* | GP494 | 10 | 800 |
| *rec12-122*[b] | GP578 | <29 | 730 |
| *rec12-140*[b] | GP682 | <47 | 1400 |
| *rec12-141*[b] | GP683 | <62 | 900 |
| *rec12-142*[b] | GP684 | <34 | 1100 |
| | | *rec15-124* | *rec6-103* |
| *rec15-124*[b] | GP569 | <250[e] | 2800 |
| *rec15-127*[b] | GP631 | <27 | 1600 |
| *rec15-132* | GP641 | <12 | 1700 |

Crosses were $h^-$ *ade6-52 rec* × $h^{90}$ *ade6-M26 ura4-294 rec*. The $h^-$ *ade6-52 rec* strains were those in Table 2. The $h^{90}$ *ade6-M26 ura4-294 rec* strains are the Ura$^-$ segregants of the mutagenized derivatives of strain GP66 (see MATERIALS AND METHODS). For entries with no Ade$^+$ colonies the upper limit of the recombinant frequency was calculated by assuming three Ade$^+$ colonies in the sample plated.

[a] These alleles and strains were described by PONTICELLI and SMITH (1989). *rec-106* is hereby designated *rec11-106*, and *rec-113*, *rec11-113*.

[b] Within each complementation group, alleles noted were isolated from the same mutagenized culture and thus may not be independent isolates (see text).

[c] Repetition of the cross gave a recombinant frequency of 113 Ade$^+$/$10^6$ viable spores.

[d] Heterothallic-by-heterothallic crosses with this allele gave lower recombinant frequencies (Table 7; PONTICELLI and SMITH 1989).

[e] Due to consistently low spore yields, the recombinant frequency could not be more accurately determined. Heterothallic derivatives of the *rec15-124* strain gave a higher spore yield, allowing a more sensitive frequency determination (see Tables 2 and 4).

For ease of testing the *rec* genotype of haploid segregants in test crosses (see MATERIALS AND METHODS), both parental strains contained the *ade6-52* mutation. The *mat2-B102* strain used (GP605) also carried mutations in genes on chromosome *I* (*leu2*) and chromosome *II* (*his5*). For each *rec* gene we constructed an $h^-$ strain that would form a nonsporulating diploid with the *mat2-B102* strain. These strains contained, in addition to a mutation in the *rec* gene being mapped, mutations in genes on chromosome *I* (*pro2*) and chromosome *III* (*ura4*). We scored haploid segregants from at least four independent diploids for each *rec* gene.

Diploids were formed, and auxotrophic segregants were isolated during mitotic growth and analyzed. The segregants were scored for *rec* and the various auxotrophies by replica-plating. To minimize mitotic recombination the diploid strains were not grown extensively before haploidization. Thus, we expected each *rec* mutation to show linkage to one of the auxotrophies. The results of this analysis are presented in Table 6. For each pair of markers, the segregants were classified into four types–P1, P2, NP1 and NP2 described in the legend to Table 6–and contingency Chi square analysis was performed on the resulting numbers of segregants. We have presented only the data for the segregation of each *rec* mutation with the three auxotrophies; the auxotrophies (except for *leu2* and *pro2*, both on chromosome *I*) showed the expected independent segregation from each other (data not shown). For the chromosome *I* segregation, we have presented only the *leu2* segregation. Since the *leu2* and *pro2* mutations in the diploids came from different parents, we have included in our analysis only those segregants which showed the parental configurations of these mutations (Leu$^+$ Pro$^-$ or Leu$^-$ Pro$^+$). We used the presence of a nonparental phenotype of these two markers (Leu$^+$ Pro$^+$ or Leu$^-$ Pro$^-$) in the segregants to indicate either a mitotic recombination event or diploidy/aneuploidy. Most of those nonparental segregants were of the Leu$^+$ Pro$^+$ type, and when examined under a microscope appeared to be diploid (data not shown). The low frequency (less than 1%) of Leu$^-$ Pro$^-$ segregants suggested that mitotic recombination did not occur at a significant rate.

In Table 6, the number of segregants of each type with respect to *rec* and the given auxotrophy is shown. For each *rec*, segregation with one auxotrophy had a Chi square value significantly higher than the other two, indicating that segregation was not random. In this way, we assigned each *rec* gene, with the exception of *rec13* and *rec17*, to a chromosome as indicated in the last column of Table 6. The *rec* phenotypes of the *rec13* and *rec17* haploids were not clear enough in the cross-stamp test to allow an accurate scoring.

**Meiotic linkage of some *rec* genes to other known genes:** Heterothallic derivatives of the $h^{90}$ *rec15-124* mutant were rarely obtained from meiotic crosses with an $h^-$ *rec*$^+$ strain. We found that the *rec15* mutation was meiotically linked to the *mat1* locus. In twenty spores isolated from heterothallic crosses ($h^+$ *ade6-M26 rec15-124* × $h^-$ *ade6-M26* or $h^-$ *ade6-52 rec15-124* × $h^+$ *ade6-52*) no recombinants between *mat1* and

**TABLE 4**

**Comparison of *M26* and *M375* recombinant frequencies in *rec* mutant strains**

| Mutation | GP strains crossed | Ade$^+$ recombinants/$10^6$ viable spores *M375 × 52* Expt 1 | Expt 2 | GP strains crossed | Ade$^+$ recombinants/$10^6$ viable spores *M26 × 52* Expt 1 | Expt 2 |
|---|---|---|---|---|---|---|
| *rec*$^+$ | 6, 13 | **320** (369) | **330** (424) | 24, 13 | **2000** (357) | **2900** (168) |
| *rec12-117* | 579, 497 | **2** (2) | **2** (2) | 592, 497 | **2** (1) | **2** (2) |
| *rec13-119* | 596, 498 | **27** (62) | **35** (9) | 593, 498 | **260** (265) | **330** (627) |
| *rec14-120* | 581, 499 | **<7** (0) | **<56** (0) | 599, 499 | **2** (2) | **<24** (0) |
| *rec15-124* | 597, 571 | **1** (1) | **6** (1) | 594, 571 | **1** (1) | **4** (1) |
| *rec16-125* | 598, 572 | **<3** (0) | **<38** (0) | 595, 572 | **23** (21) | **33** (4) |
| *rec17-112* | 731, 606 | **43** (16) | **92** (46) | 687, 606 | **420** (160) | **770** (477) |
| *rec18-138* | 732, 659 | **94** (10) | **<56** (0) | 661, 659 | **300** (95) | **650** (339) |
| *rec19-139* | 685, 660 | **16** (18) | **45** (77) | 662, 660 | **550** (377) | **410** (201) |
| *rec20-144* | 730, 728 | **45** (115) | **66** (140) | 729, 728 | **440** (387) | **430** (243) |
| *rec21-143* | 692, 690 | **33** (89) | **130** (253) | 691, 690 | **210** (338) | **570** (550) |
| *swi5-134* | 586, 583 | **89** (9) | | 584, 583 | **390** (70) | |

Crosses were $h^+$ *ade6-M26* or *M375 rec* × $h^-$ *ade6-52 rec*. Numbers in parentheses indicate Ade$^+$ colonies counted. At least 200 colonies were counted to determine viable spores. The *ade6-M375* strains were constructed by crossing GP6 ($h^+$ *ade6-M375*) and the $h^-$ *ade6-52 rec* strains in Table 2, with the exception of GP597. Due to the meiotic linkage of *rec15* and *mat*, GP597 was constructed from the cross of GP43 ($h^-$ *ade6-M375 leu2*) and GP594 ($h^+$ *ade6-M26 rec15-124*). The *ade6-M26* and *ade6-52* strains were those in Table 2.

**TABLE 5**

**Effect of certain *rec* mutations on *ade6* and *ura4* intragenic and *pro2-arg3* intergenic meiotic recombination**

| *rec* | GP strains crossed | Prototrophic recombinants/$10^6$ viable spores Ade$^+$ I | Ade$^+$ II | Ade$^+$ III | Ura$^+$ I | Ura$^+$ II | Ura$^+$ III | Pro$^+$ Arg$^+$ I | Pro$^+$ Arg$^+$ II |
|---|---|---|---|---|---|---|---|---|---|
| + | 363,369 | 1400 | 1500 | 1300 | 190 | 220 | 340 | 71,000 | 55,000 |
| *6-103* | 364,370 | — | — | 7 | — | — | <21 | — | 6,700 |
| *8-110* | 436,427 | — | — | 11 | — | — | <22 | — | 59,000 |
| *9-104* | 366,372 | — | — | 550 | — | — | 34 | — | 13,000 |
| *12-117* | 500,503 | <18 | <20 | — | <18 | <20 | — | 12,000 | — |
| *13-119* | 501,504 | 150 | 160 | — | 40 | 50 | — | 25,000 | — |
| *14-120* | 502,505 | <120 | <20 | — | <120 | <20 | — | 16,000 | — |

I, II and III are separate experiments. The strains crossed are described in Table 1 and have the genotypes $h^+$ *ade6-M26 ura4-294 arg3-124 rec* and $h^-$ *ade6-52 ura4-595 pro2-1 rec*. — = not determined.

*rec15* were observed. This result confirmed our assignment of the *rec15* gene to chromosome *II*. The *rec6* gene is meiotically linked to the *pat1* gene (P. SZANKASI, personal communication), and *rec11* to *ade6* (P. SZANKASI, A. S. PONTICELLI and G. R. SMITH, unpublished observations); both linkages are approximately 1–2 cM and confirm our assignment of these genes to chromosome *II* and *III*, respectively.

## DISCUSSION

Prior to this study, the number of genes known to be involved in meiotic recombination in *S. pombe* was low. Mutations in seven genes, *rec6–rec11* (PONTICELLI and SMITH 1989) and *swi5* (SCHMIDT, KAPTIZA and GUTZ 1987), reduce intragenic recombination at the *ade6* locus from 3- to 1000-fold. Because only one or two mutations in each of these genes had been obtained, we reasoned that the pool of *rec* genes amenable to this screen must be larger than this and undertook a more extensive search for *rec* mutants, using the screen of PONTICELLI and SMITH (1989).

**Isolation of mutants:** We have assigned thirty recessive mutations that decrease meiotic intragenic recombination to 17 complementation groups. Strains carrying these mutations vary greatly in degree of deficiency, although within a complementation group, multiple alleles usually behaved similarly. Mutations in five of the six genes identified by PONTICELLI and SMITH (1989) reduce recombination by at least 100-fold, and our search identified four additional complementation groups that were as severely affected. We also isolated 20 additional alleles of the groups with reductions of 100-fold or greater but found no additional alleles of the less deficient groups. A summary of our results on the 10 new complementation groups and *swi5*, as well as the results of PONTICELLI and SMITH (1989) on *rec6–rec11*, is presented in Table 7. The distribution of alleles for the strongest *rec* genes suggests that saturation is being approached and that only a few more genes of this type are likely to be identified by this screen.

The screen we have used to isolate *rec* mutations

**TABLE 6**

**Assignment of *rec* genes to chromosomes by mitotic segregation**

| Genes | | No. of segregants | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | P1 | P2 | NP1 | NP2 | $\chi^2$ | Chromosome | |
| *rec6* | *leu2* | 14 | 40 | 25 | 26 | <0.1 | | |
| | *his5* | 20 | 52 | 19 | 5 | 19.6 | *II* | |
| | *ura4* | 20 | 36 | 19 | 30 | 0.1 | | |
| *rec7* | *leu2* | 9 | 35 | 14 | 13 | 0.6 | | |
| | *his5* | 12 | 27 | 11 | 21 | 0.2 | | |
| | *ura4* | 23 | 46 | 0 | 2 | 58.5 | *III* | |
| *rec8* | *leu2* | 6 | 92 | 30 | 21 | <0.1 | | |
| | *his5* | 36 | 110 | 0 | 3 | 128.9 | *II* | |
| | *ura4* | 14 | 64 | 22 | 49 | <0.1 | | |
| *rec9* | *leu2* | 54 | 47 | 5 | 4 | 73.5 | *I* | |
| | *his5* | 24 | 18 | 35 | 33 | 5.4 | | |
| | *ura4* | 20 | 32 | 39 | 19 | <0.1 | | |
| *rec10* | *leu2* | 19 | 51 | 2 | 4 | 46.0 | *I* | |
| | *his5* | 9 | 30 | 12 | 25 | <0.1 | | |
| | *ura4* | 10 | 29 | 11 | 26 | <0.1 | | |
| *rec11* | *leu2* | 18 | 38 | 36 | 11 | 1.0 | | |
| | *his5* | 13 | 33 | 41 | 16 | 0.6 | | |
| | *ura4* | 54 | 46 | 0 | 3 | 87.8 | *III* | |
| *rec12* | *leu2* | 14 | 70 | 0 | 0 | 76.9 | *I* | |
| | *his5* | 2 | 51 | 12 | 19 | 0.5 | | |
| | *ura4* | 7 | 47 | 7 | 23 | 0.8 | | |
| *rec14* | *leu2* | 22 | 12 | 99 | 7 | 2.4 | | |
| | *his5* | 120 | 19 | 1 | 0 | 12.4 | *II* | |
| | *ura4* | 59 | 9 | 62 | 10 | <0.1 | | |
| *rec15* | *leu2* | 31 | 47 | 68 | 17 | 0.2 | | |
| | *his5* | 97 | 63 | 2 | 1 | 147.0 | *II* | |
| | *ura4* | 41 | 40 | 58 | 24 | 0.1 | | |
| *rec16* | *leu2* | 7 | 72 | 14 | 45 | <0.1 | | |
| | *his5* | 18 | 100 | 3 | 17 | 44.0 | *II* | |
| | *ura4* | 14 | 48 | 7 | 69 | 0.2 | | |
| *rec18* | *leu2* | 28 | 9 | 6 | 6 | 6.9 | *I* | |
| | *his5* | 14 | 5 | 20 | 10 | 1.8 | | |
| | *ura4* | 19 | 6 | 15 | 9 | <0.1 | | |
| *rec19* | *leu2* | 36 | 102 | 8 | 2 | 99.0 | *I* | |
| | *his5* | 14 | 68 | 30 | 36 | <0.1 | | |
| | *ura4* | 27 | 56 | 17 | 48 | 2.3 | | |
| *rec20* | *leu2* | 33 | 129 | 8 | 3 | 111.4 | *I* | |
| | *his5* | 8 | 80 | 33 | 52 | 4.6 | | |
| | *ura4* | 25 | 70 | 16 | 62 | 1.9 | | |
| *rec21* | *leu2* | 11 | 24 | 15 | 10 | 0.6 | | |
| | *his5* | 23 | 25 | 3 | 9 | 20.3 | *II* | |
| | *ura4* | 9 | 12 | 17 | 22 | 4.2 | | |
| Matings were: | $h^{90}$ | *mat2-B102* | *ade6-52* | *leu2-120* | + | *his5-303* | + | + |
| | $\times h^-$ | + | *ade6-52* | + | *pro2-1* | + | *ura4-595* | *rec* |

The $h^{90}$ parent was GP605, and the $h^-$ parents were derived from crosses between the $h^-$ *ade6-52 rec* strains in Table 2 and GP350 ($h^+$ *pro2-1 ura4-595*), except for the *rec8* strain, GP699, which is described in Table 1. Haploid mitotic segregants were obtained and analyzed as described in MATERIALS AND METHODS. P1 (parental) segregants had the genotype of the $h^{90}$ parent, GP605, with respect to *rec* and each auxotrophy. P2 segregants had the genotype of the $h^-$ parent. NP1 (nonparental) and NP2 segregants had the non-parental genotypes, NP1 being *rec*$^+$ and NP2 being *rec*. Contingency $\chi^2$ values were calculated according to STRICKBERGER (1968). The *leu2* and *pro2* genes are located on chromosome *I*, *his5* is on chromosome *II*, and *ura4* is on chromosome *III* (KOHLI 1987). Only auxotrophs containing either a leucine or proline auxotrophy, but not both, were included in this study. Other auxotrophs were diploid or aneuploid as revealed by microscopic examination of the cells.

may be biased in several ways. First, the initial selection for candidates relies in part on a qualitative visual inspection for spots with few white colonies; certainly this is most dramatic for the most deficient mutants, which would be more likely to be chosen than would a less striking mutant. The preponderance of strongly recombination-deficient mutations isolated suggests such a bias. Second, mutations that affect mitotic growth or spore viability or that cause meiotic lethality would also not have been isolated, as the mutated

**TABLE 7**

**Summary of *rec* mutant characterization**

| Gene | No. of alleles | Meiotic recombination frequency at *ade6*: Plasmid × chromosome Ade$^+$/10$^5$ viable spores *M26 × 469* | Meiotic recombination frequency at *ade6*: Chromosome × chromosome Ade$^+$/10$^6$ viable spores *M26 × 52* | Meiotic recombination frequency at *ade6*: Chromosome × chromosome Ade$^+$/10$^6$ viable spores *M375 × 52* | DNA damage sensitivity | Chromosome | Meiotic linkage |
|---|---|---|---|---|---|---|---|
| *rec*$^+$ | | 25 | 2500 | 330 | | | |
| Class I | | | | | | | |
| *rec6* | 4 | <0.5 | 4 | 3 | | *II* | *pat1*[a] |
| *rec7* | 4 | <0.5 | 2 | 2 | | *III* | |
| *rec8* | 4 | 0.4 | 4 | 5 | | *II* | |
| *rec12* | 5 | 0.5 | 2 | 2 | | *I* | |
| *rec14* | 1 | <0.8 | 2 | <7 | | *II* | |
| *rec15* | 3 | 0.1 | 3 | 3 | | *II* | *mat1* |
| Class II | | | | | | | |
| *rec10* | 5 | 0.8 | 30 | 5 | | *I* | |
| *rec11* | 5 | 0.6 | 12 | 4 | | *III* | *ade6*[b] |
| *rec16* | 1 | 0.6 | 28 | <3 | | *II* | |
| Class III | | | | | | | |
| *rec9* | 1 | 5 | 1000 | 77 | MMS, UV | *I* | |
| *rec13* | 1 | 7.5 | 300 | 31 | | | |
| *rec17* | 1 | 6.0[c] | 600 | 68 | MMS | | |
| *rec18* | 1 | 10 | 480 | 94 | | *I* | |
| *rec19* | 1 | 4.4 | 480 | 30 | MMS | *I* | |
| *rec20* | 1 | <0.8 | 440 | 55 | | *I* | |
| *rec21* | 1 | <0.1 | 390 | 82 | | *II* | |
| *swi5* | 3 | | 390 | 89 | UV | *II* | |

Recombinant frequencies and DNA damage sensitivities for *rec6–rec11* have been reported previously (PONTICELLI and SMITH 1989). For *rec*$^+$, *swi5* and *rec12–rec21* the meiotic *ade6* interchromosomal recombinant frequencies are the averages of the two experiments of Table 4. The chromosome assignments are summarized from Table 6 (except for *swi5*). Information on the *swi5* alleles was reported by GUTZ and SCHMIDT (1985) and KOHLI (1987). Other data were determined as described in MATERIALS AND METHODS. The number of alleles for each *rec* gene includes those reported previously (PONTICELLI and SMITH 1989).

[a] P. SZANKASI, personal communication.

[b] P. SZANKASI, A. S. PONTICELLI and G. R. SMITH (unpublished data).

[c] PONTICELLI and SMITH (1989); *rec-112* has been designated *rec17-112*.

strain was required to mate and sporulate, and the spores to germinate and grow well. Because *swi5* mutations also affect mating-type switching (GUTZ and SCHMIDT 1985), such mutations may not have passed the requirement for self-mating imposed by the screen, and indeed none were isolated. Because of these requirements, there may be unidentified *rec* genes that have additional roles in other processes. Finally, only those mutations which affect plasmid-by-chromosome recombination would have been detected. Thus, mutations that affect only chromosome-by-chromosome interactions, such as pairing, would have been overlooked.

Dominant mutations were not analyzed further but represented approximately half of the initial candidates. PONTICELLI and SMITH (1989) reported a dominant mutation, *rec-101*, which was not linked to *ade6*. From our analysis, it was clear that whereas some dominant mutations were additional mutations in the *ade6* gene, others were not and may be of future interest.

**Classes of mutants:** PONTICELLI and SMITH (1989) placed *rec6–rec11* into two classes based on their effect on the stimulation of recombination by the *M26* recombination hotspot. We believe that the *rec* phenotypes warrant a third class. In a *rec*$^+$ background, a cross in which one parent contains the hotspot *ade6-M26* shows a 10–15-fold higher recombinant frequency than a comparable non-hotspot *ade6-M375* cross (GUTZ 1971). Class I *rec* mutants show the same low level of recombination in either hotspot or non-hotspot crosses (Table 7). In these crosses a few Ade$^+$ recombinants were seen. This residual recombination was above the reversion frequency of the three *ade6* alleles used (see MATERIALS AND METHODS). We believe that this low basal level of recombination is produced by a pathway independent of the *rec* genes studied here. This pathway, which we shall call the basal pathway, is not stimulated by the *M26* hotspot. Mitotic recombination is also not stimulated by the *M26* hotspot (PONTICELLI, SENA and SMITH 1988) and may proceed by the basal pathway. The high level *rec*-dependent pathway is *M26*-stimulated and appears to be meiotic-specific, since the *rec* mutants tested to date do not have significantly altered mitotic recombination rates (K. L. LARSON and N. HOLLINGSWORTH, personal communications).

We have defined three classes of *rec* mutants with respect to their effects on the *rec*-dependent pathway. Class I mutants may be completely deficient for the *rec*-dependent pathway, but like all *rec* mutants they retain the basal pathway. Class II mutations strongly reduce the *rec*-dependent pathway but do not abolish it; recombination by this pathway is detectable only in the presence of the *M26* hotspot. The use of the *M26* hotspot in the screening of these mutants provided a means of distinguishing class II mutants from class I mutants. Class III mutants are approximately 3–10-fold reduced for the *rec*-dependent pathway; in each mutant hotspot and non-hotspot recombination are equivalently reduced. The mutant phenotypes suggest that each class I, and possibly class II, gene may be essential for recombination through the *rec*-dependent pathway, whereas class III genes are not. We further infer that both hotspot and non-hotspot recombination proceed by the *rec*-dependent pathway.

We have assigned *rec12*, *rec14* and *rec15* to class I; mutations in these genes, as well as those in *rec6*, *rec7* and *rec8*, all reduce *ade6* hotspot recombinant frequencies approximately 1000-fold. We have placed *rec16* in class II with *rec10* and *rec11*. We have reclassified *rec9* to class III, along with our remaining new mutations (*rec13*, *rec17*, *rec18*, *rec19*, *rec20* and *rec21*) and *swi5* (Table 7). We found the *rec* genes in each class to be distributed among the three chromosomes of *S. pombe* (Table 6). The meiotic mapping data for two class I genes, *rec6* and *rec15*, show that, although mutations in the two genes have similar phenotypes and the genes are both located on chromosome *II*, they are meiotically unlinked.

Since our screen used the *M26* hotspot, we might have expected to recover a mutation specific for hotspot recombination. All of the mutations tested affected both hotspot and non-hotspot recombination, although in some cases to different extents (Table 4). A hotspot-specific mutant might reduce hotspot recombinant frequencies to those of non-hotspot recombinant frequencies but not alter the latter. As discussed above, mutations that decreased recombination only 10-fold might not be as easily detected in this screen as those that more drastically reduced it, and thus the screen may be biased against recovery of such hotspot-specific mutants. Although we isolated seven class III mutants, only one allele of each gene was obtained, which supports our suggestion that the screen was not as effective at identifying such mutants. Alternatively, a hotspot-specific protein might also play an integral role in meiosis, such that a mutation in the gene encoding such a protein would be meiotically lethal, or the mutation might affect mitotic growth and have been overlooked. A third possibility is that such a protein might be involved in general recombination, so that a mutation would affect both hotspot and non-hotspot recombination. A bacterial analog is the RecBCD enzyme, which recognizes the recombination-stimulating sequence Chi, but mutations in *recB* or *recC* reduce recombination in the absence as well as the presence of Chi (SMITH 1988). Thus, one of the *rec* genes we have identified may encode a hotspot-specific protein.

**Effect of *rec* mutations on other recombination events:** We sought mutants deficient in chromosome-by-chromosome recombination and used plasmid-by-chromosome recombination in the initial screen. For the most part, there is a good correlation between the degree of reduction of plasmid-by-chromosome recombination and that of chromosome-by-chromosome recombination (Table 7). This correlation indicates that the pathway for plasmid-by-chromosome recombination shares many components with the chromosome-by-chromosome pathway. To isolate the heterothallic derivatives of each mutant, we scored chromosome-by-chromosome recombination. Thus, a mutant deficient only in plasmid-by-chromosome recombination would not have been analyzed further. We did isolate 13 recessive mutants for which heterothallic derivatives were not obtained. These mutants may be deficient only in plasmid-by-chromosome recombination. Alternatively, their *rec* mutations may be tightly linked to either *mat1* or *ade6*, making isolation of the desired derivatives difficult. Of these 13 mutants, 6 had plasmid-by-chromosome recombinant frequencies comparable to that of class I and class II mutants (data not shown); it may be fruitful to analyze these mutants further.

We examined the effect of several of the *rec* mutations on meiotic recombination at intervals other than the *ade6* locus (Table 5). *rec6*, *rec8* and *rec9*, described by PONTICELLI and SMITH (1989), as well as *rec12*, *rec13* and *rec14*, were also tested for their effect on intragenic recombination at the *ura4* locus and for intergenic recombination in the *pro2-arg3* interval. PONTICELLI and SMITH (1989) reported that for the three *rec* genes tested the degree of reduction at the *ura4* locus is not as great as that at *ade6* when the *ade6-M26* hotspot is involved. However, if *ura4* recombination is compared to non-hotspot recombination at *ade6*, the degree of reduction is more nearly equal. In the case of the class I mutation (*rec7*) tested by PONTICELLI and SMITH (1989), both *ura4* and *ade6* non-hotspot recombination are reduced about 70-fold. For the class I mutations tested in this study (*rec6*, *rec8*, *rec12* and *rec14*) no $Ura^+$ recombinants were obtained; however, *ura4* recombination was reduced at least 10-fold. The two class II mutations (*rec10* and *rec11*) tested by PONTICELLI and SMITH (1989) show a substantial difference in the reduction of $Ade^+$ and $Ura^+$ recombinant frequencies; this difference diminishes but does not disappear if non-

hotspot recombination is compared instead of hotspot. For the class III mutations analyzed here (*rec9* and *rec13*), the reduction of recombination at the *ura4* locus was comparable to the reduction at *ade6*. While the effect of these mutations on *pro2-arg3* intergenic recombination was not as dramatic, there was still an effect by all the mutations, with the exception of *rec8*. *rec8* reduced *ade6* intragenic recombination over 100-fold but had no significant effect on *pro2-arg3* intergenic recombination. For the other mutations the degree of reduction was similar to the degree of reduction of intragenic recombination; this finding supports the idea that these genes are involved in meiotic recombination throughout the genome.

We have considered four interpretations of the differential reductions in recombinant frequencies. First, some *rec* gene products may be required for intragenic recombination (presumably gene conversion) but not for intergenic recombination (presumably crossing over). The $rec8^+$ gene product, for example, may be required for gene conversion but not crossing over. CARPENTER (1987) and HASTINGS (1988) have discussed evidence that conversion and crossing over occur by distinct mechanisms.

Second, some *rec* gene products may be required for correction of base mismatches that arise when hybrid DNA is formed at the site of a mutational difference between the parental strains; mismatches at one test interval may be sensitive to the action of a particular *rec* gene product and those at another not. The *PMS1* gene product of *S. cerevisiae* is required for correction of G/G mismatches but not of C/C mismatches (BISHOP, ANDERSEN and KOLODNER 1989).

Third, a particular *rec* mutation may alter the length of hybrid DNA formed during recombination. For example, if $ade6^+$ recombinants are formed primarily when hybrid DNA covers only one *ade6* allele, then longer hybrid DNA tracts would decrease the $ade6^+$ recombinant frequency. If these tracts were shorter than the *pro2-arg3* interval, then the *pro2-arg3* recombinant frequency would be scarcely affected. In this view, hybrid DNA might be longer in *rec8* mutants.

Finally, some *rec* gene products might be more stringently required for recombination at one locus than at another. For example, the *rec8* gene product may be required at *ade6* and *ura4* but not at *pro2* or *arg3*. CATCHESIDE (1977) has reviewed the properties of *Neurospora crassa rec* gene products, which repress recombination at some loci but not at others. The possible differential locus specificity of the *S. pombe rec* gene products predicts, for example, that recombination of all *ade6* alleles, but not that of *pro2* or *arg3* alleles, would be reduced by the *rec8* mutation.

**Mitotic phenotypes:** Several findings suggest that mitotic and meiotic recombination and DNA repair may utilize different pathways and enzymes. Of the mutants isolated by this screen, only *rec9*, *rec17* and *rec19* showed sensitivity to DNA-damaging agents. In the case of *rec17* and *rec19*, that effect was slight. *rec9-104* does not significantly alter mitotic recombinant frequencies (N. HOLLINGSWORTH and K. L. LARSON, personal communications). Conversely, the *S. pombe rec1* gene affects mitotic, but not meiotic, recombination (GOLDMAN and GUTZ 1974). Additional mutations altering mitotic but not meiotic recombination have recently been reported (GYSLER-JUNKER, BODI and KOHLI 1991). Similarly, some *S. cerevisiae rad* mutants are altered in mitotic but not meiotic recombination (KUNZ and HAYNES 1981; ESPOSITO *et al.* 1984), and newly identified early meiotic *rec* mutations have no effect on mitotic recombination (MALONE *et al.* 1991). These observations support the hypothesis, discussed earlier, of a *rec*-dependent meiotic pathway and a *rec*-independent mitotic pathway.

In conclusion, the screen developed by PONTICELLI and SMITH (1989) has been used to isolate mutations in 16 complementation groups affecting meiotic recombination in *S. pombe*, bringing to 17 the number of genes thus far identified to be involved in meiotic recombination. Since the mutations most severely affecting recombination fall into a few groups with multiple alleles, we believe that we are nearing saturation for these types of mutations. Cloning the genes by complementation of the strong recombination-deficient phenotype should enable the construction of null mutations in these genes, and the determination of their nucleotide sequences will allow a physical analysis essential for understanding the role that each gene plays in meiotic recombination.

We thank JÜRG KOHLI and GINGER ZAKIAN for strains of *S. pombe*, FRED PONTICELLI for some of the mutagenized *S. pombe* cells, KAREN LARSON for helpful genetic advice, PHILIPPE SZANKASI, ANDREW TAYLOR, SUSAN HOLBECK and SUE AMUNDSEN for comments on the manuscript, KAREN LARSON and NANCY HOLLINGSWORTH for unpublished data, and KAREN BRIGHTON for sharing her word processing expertise. This work was supported by research grants GM31693 and GM32194 from the National Institutes of Health to G.R.S.

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Communicating editor: G. S. ROEDER